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Xenotransplantation of Transgenic Oligodendrocyte-Lineage Cells into Spinal Cord-Injured Adult Rats

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Spinal cord trauma is associated not only with loss of nerve cells and fibers but also with damage to oligodendrocytes and demyelination. In order to assess the potential of transplanted oligodendrocyte-lineage cells to repair the demyelination that follows spinal cord injury, we have used donor glia derived from a transgenic mouse line containing the LacZ transgene under control of the myelin basic protein promoter (16). Glia derived from fetal or neonatal transgenic mice were injected into the spinal cords of immunosuppressed adult rats at the site of an experimental traumatic lesion 1–16 days after injury. Cells expressing LacZ were identified 15–18 days later in cryosections rostral and caudal to the transplant site, most conspicuously within white matter defects. Some of these cells within the dorsal columns gave rise to ~30- to 60-μm processes, consistent with myelin segments, which are oriented parallel to the fiber tract. Glial transplantation may thus be a feasible means of replacing damaged host oligodendrocytes with donor oligodendrocyte-lineage cells capable of reforming myelin and potentially restoring function lost as a result of demyelination associated with spinal cord injury. © 1997 Academic Press

INTRODUCTION

Previous studies have shown that allografts of oligodendrocyte-lineage cells will form myelin in the central nervous system (CNS) of congenitally myelin-deficient animals (17, reviewed in 4) in developing normal CNS (19, 26) and in demyelinated lesions of the CNS (3, 12, 15, 27). Mouse glial cell xenografts into rat CNS have also succeeded in immunosuppressed hosts (12, 23). With both allografts and xenografts, the best results are produced not by adult oligodendrocytes but rather by precursor cells obtained from fetal or neonatal donors (22, 27, 28).

Spinal cord injury has been shown to result not only in damage to neural elements but also in a significant amount of demyelination, which has been documented in an extensive series of studies (5–11). Glial transplantation here as well could potentially result in some degree of remyelination beyond what occurs spontaneously (10) and might therefore lead to additional restoration of function. Transplanted glia could also serve to form myelin around regenerated axons.

Studies of embryonic rat spinal cord transplanted into surgical lesions of adult rat spinal cord have shown survival, growth, and maturation of the transplants as well as extensive myelination of the donor tissue (20). Thus the environment of the lesioned adult spinal cord does not preclude myelin formation. In impact lesions, however, the success of transplantation could be affected by the conspicuous inflammation that follows this form of trauma (7, 8), which could damage not only endogenous but also exogenous oligodendrocyte-lineage cells. The environment of the traumatized spinal cord could thus compromise the survival of transplanted oligodendrocytes or their ability to form myelin.

In order to investigate the feasibility of glial transplantation after spinal cord injury, we injected mouse oligodendrocyte-lineage cells into experimental traumatic lesions of rat spinal cord caused by controlled impact (2, 18). Because of the presence of residual host-derived myelin-forming cells in the traumatized spinal cord, it would be difficult to distinguish them from donor myelin-forming cells without a marker. For this reason, we used donor cells obtained from a transgenic mouse line which carries the LacZ (bacterial galactosidase) gene under control of the MBP promoter (14, 16).

Oligodendrocyte-lineage cells from these animals do not express LacZ constituitively. Thus O2A progenitors derived from neonatal donors would be $LacZ^-$ and would become $LacZ^+$ only after differentiating to the stage at which myelin basic protein (MBP) is expressed. The marker, therefore, does not identify all donor cells or even all oligodendrocyte-lineage donor cells, but only

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those oligodendrocytes that have matured sufficiently to transcribe the MBP gene.

MATERIALS AND METHODS

Female Long–Evans hooded rats weighing ~300 g were anesthetized with pentobarbital (45 mg/kg ip), and laminectomy was performed at the T9–10 level. During surgery and postoperatively, body temperature was maintained at 37 ± 1°C by means of a heating pad. Spinal cord injury was inflicted by the NYU Impactor (2, 18) using a 10-g rod dropped from a height of 12.5 mm. Muscle and skin were then closed over the laminectomy site. This trauma produces ~50–70% loss of white matter at the impact site by 6 weeks after injury. The lesion is centered in the central gray matter and extends concentrically outward to involve white matter and rostrocaudally about 5 mm. Rats injured by a 12.5-mm weight drop suffer immediate paraplegia but typically recover locomotor function within 4 weeks, including ability to support weight and stepping, but without forelimb-hindlimb coordination. The bladders are paralyzed, and the rats require twice daily bladder expression until automatic micturation recovers. In order to prevent urinary tract infection, the rats are treated with an antibiotic (Keflin, 30 mg/day for 7 days after injury). On the BBB scale (1, 2), for assessing behavioral performance, the rats typically achieve a score of 10–11 at 2 weeks and 12–13 at 3 weeks out of a total scale of 0–21.

Oligodendrocyte-lineage cells were obtained from MβP5 transgenic mouse embryo (E16–20) or neonatal (P1–2) brains (16). (Transgenic breeders were kindly provided by Dr. R. Lazzarini.) The tissue was minced, dissociated with trypsin, and separated on a Percoll gradient according to methods used previously, which yield mixed glial cultures free of neurons (22). MβP mouse cells contain multiple copies of a transgene consisting of the LacZ reporter under control of MBP promoter/enhancer elements. Thus, transgenic cells of the oligodendrocyte lineage express LacZ only after they have differentiated to the point of MBP expression. The transgene is not expressed by astrocytes or microglia.

Glia obtained from the transgenic mice were either cultured in a CO_2 incubator for 8–20 days *in vitro* (DIV) or, in one case, purified, stored overnight at 4°C, and then used directly. Cells to be transplanted were suspended in L15 culture medium. An aliquot was mixed with trypan blue and the concentration of live cells determined from counts in a hemocytometer. (Any cells stained with trypan blue were excluded from the count.) Cell concentration was adjusted to ~5×10^7 cells/ml, and 0.02 ml was then injected into the spinal cord of anesthetized rats (1–16 days postinjury) through the original laminectomy site, directed at the epicenter of the trauma. Cyclosporine (10 mg/kg/ip) was administered on the day of transplant and daily thereafter.

Fifteen to eighteen days later, the rats were reanesthetized and fixed by vascular perfusion with 2% paraformaldehyde in 0.1 *M* cacodylate buffer (pH 7.3) containing 2 m*M* $MgCl_2$ and 1.25 m*M* EGTA. Previous studies have shown this to be the approximate time at which myelin formation by transplanted glia has plateaued and can be demonstrated reliably (22, 23). Spinal cords were divided into segments, infiltrated with sucrose, frozen, and sectioned either transversely or horizontally at ~30–50 μm. Sections were processed in X-gal reaction solution overnight at neutral pH (16) and then mounted for examination and photography. Cells expressing bacterial β-galactosidase are stained blue by this method. The mammalian enzyme, which is active at a much lower pH, produces no detectable reaction product when incubated under these conditions.

Positive controls consisted of spinal cord sections from a transgenic mouse, processed together with the experimental sections in each staining run, to show that the reaction conditions and reagents were adequate to yield reaction product. A negative control consisted of a rat spinal cord subjected to equivalent trauma and not given a transplant, but incubated in X-gal reaction solution 21 days later.

Transverse cryosections were cut from four successive lengths of spinal cord, each ~2–3 mm long, extending rostrally from the lesion site and from four successive lengths extending caudally. Some segments were sectioned horizontally. After incubation in the X-gal reaction solution, sections were scanned for blue-stained cells and photographed using a red filter. For each animal, the number of stained cells was counted in a randomly chosen single transverse section taken at each of the eight spinal cord levels adjacent to the lesion, and the counts were summed.

RESULTS

Spinal cord sections from our negative control showed no stained cells after incubation in the X-gal reaction solution (Fig. 1A), indicating that under the conditions we used, mammalian galactosidases in host macrophages or other host cells do not generate detectable reaction product from the X-gal reaction mixture. This negative result is consistent with results we have obtained in control animals from other studies (unpublished) in which traumatized or untraumatized rat spinal cords have been exposed to X-gal reaction mixture and also fail to show reaction product due to endogenous enzyme. In contrast, sections from $LacZ^+$ adult mice from our transgenic breeding colony, used as positive controls, showed strong staining in all white matter tracts, as well as scattered stained cells in gray

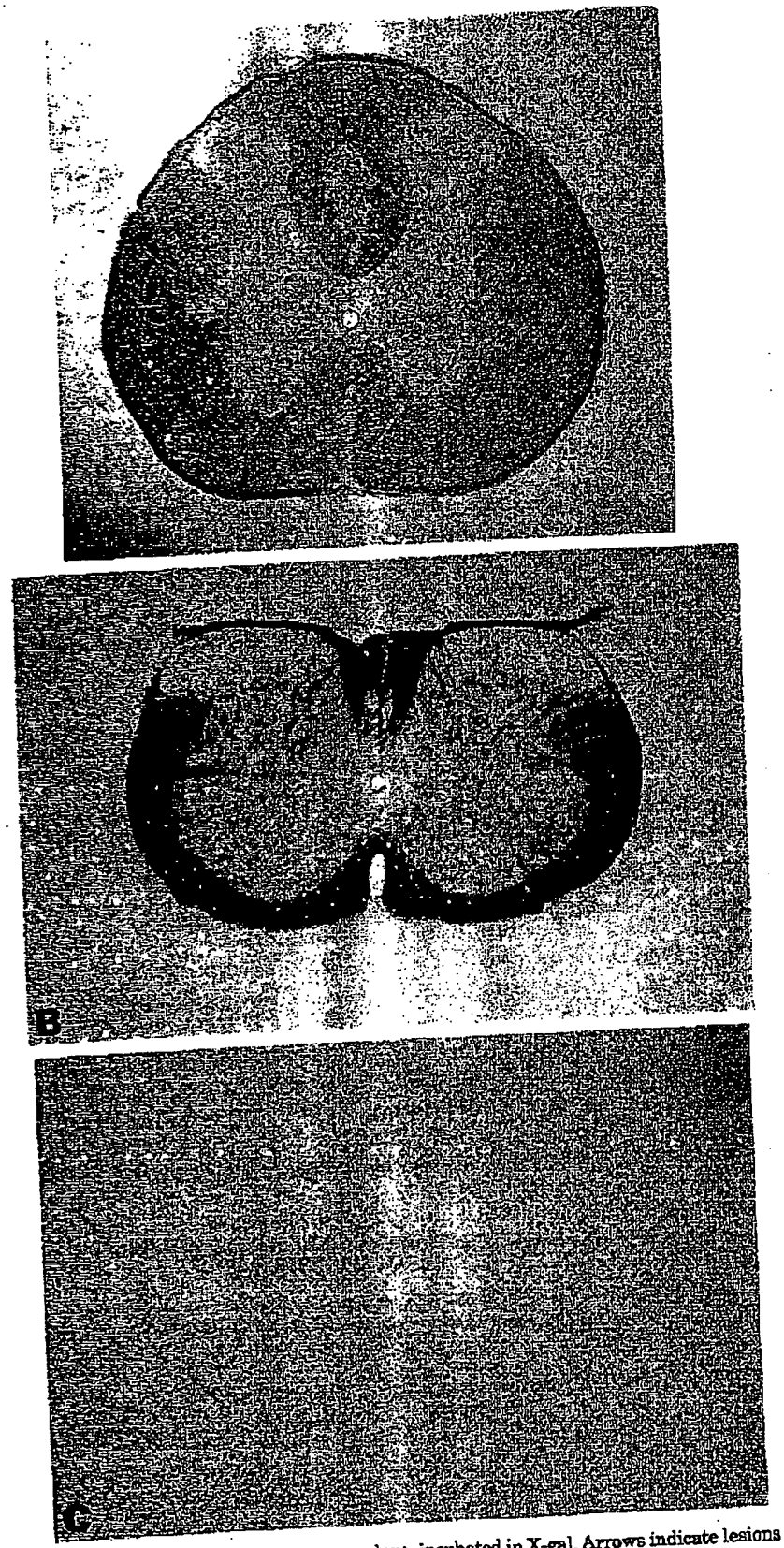

FIG. 1. (A) Negative control. Spinal cord-injured rat, no transplant, incubated in X-gal. Arrows indicate lesions within dorsal and lateral columns. Note absence of reaction product. (B) Positive control. Transgenic mouse spinal cord; incubation in X-gal results in dense staining of white matter and of stained cells in gray matter. (C) Same as B without X-gal incubation to show density of unstained myelin under comparable optical conditions

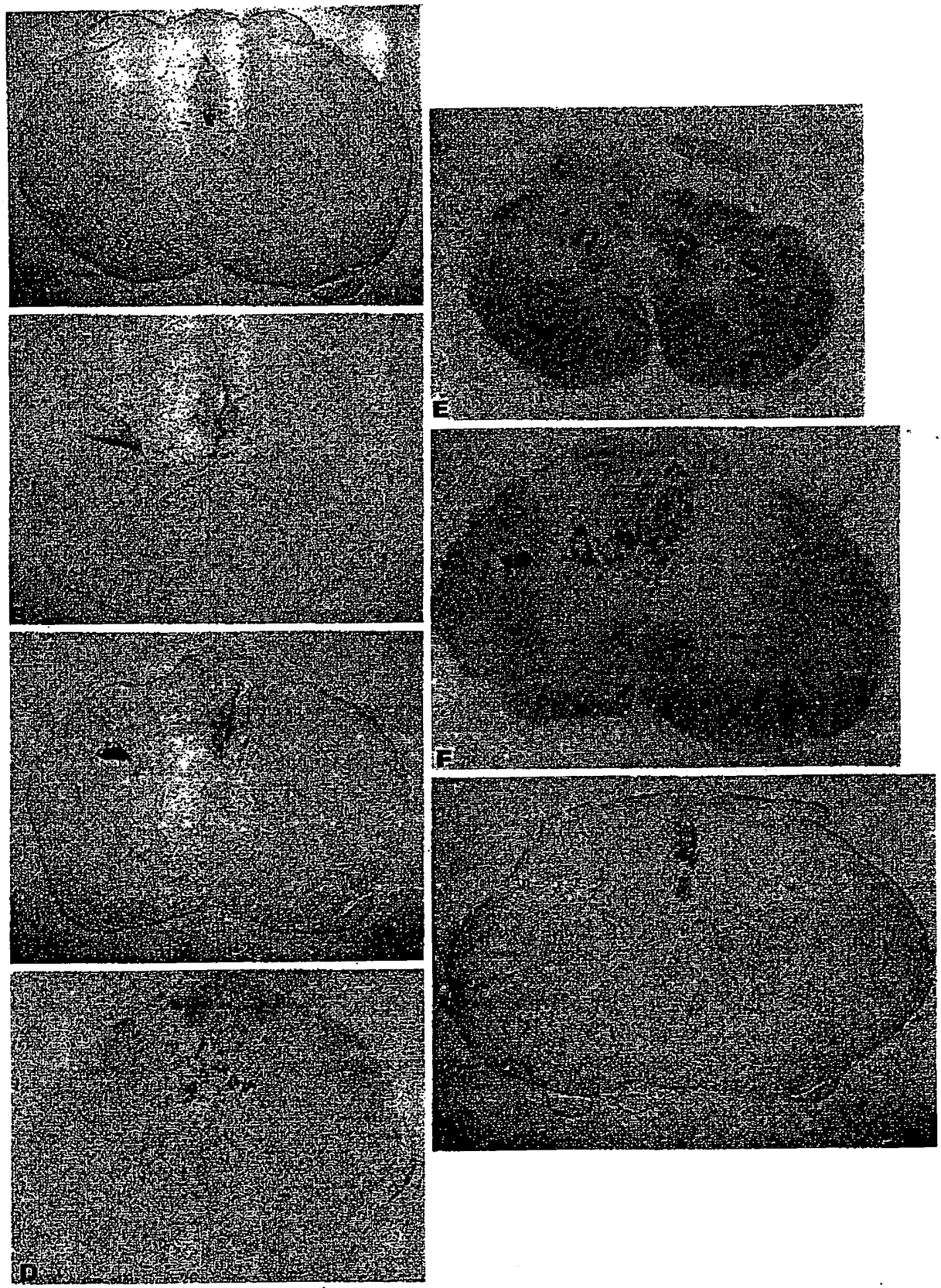

FIG. 2. Sections incubated in X-gal. From successive 2- to 3-mm lengths (A–D rostral and E–G caudal to injury site). Transgenic glial transplant performed 13 days after injury. Fixed 17 days after transplant. Clusters of stained cells are visible primarily in dorsal and lateral white matter regions at all levels shown. In several cases (e.g., at arrow) a lucent area of defective myelin surrounds the cluster of stained cells. Stained cells are also scattered within gray matter (cf. Fig. 1B).

TABLE 1

Summary of Findings

Interval, trauma to transplant (days)	Donor source (mouse)	Interval, transplant to fixation (days)	Cell count[a]
1	P1 + 10 DIV	18	60
1	P1 + 10 DIV	18	29
4	P1 + 13 DIV	15	55
6	E19/20 + 18 DIV	16	41
9	E17/18 + 4°C, 18 h	17	6
13	P1/2 + 20 DIV	17	>400
16	E16–18 + 8 DIV	18	69
[b]	None (control)	[b]	0

[a] Cell count = $\Sigma(L_1 \ldots L_8)$, where L_x represents the number of stained cells from a randomly chosen transverse section at level x. Levels 1–8 consist of the four 2- to 3-mm lengths of spinal cord just rostral to the level of the lesion plus the four just caudal to that level.
[b] Rat fixed 21 days after trauma.

matter (Fig. 1B), compared with adjacent sections not exposed to the reaction solution (Fig. 1C).

Of the six rats that received transplants of cultured glia grown from fetal or neonatal transgenic mice, five, transplanted at 1, 4, 6, and 16 days postinjury, showed moderate numbers of stained cells at multiple spinal cord levels, and the remaining one, transplanted at 13 days, showed very extensive staining (Fig. 2). The rat injected with donor cells that had not first been cultured showed few stained cells. Table 1 summarizes these findings.

In all of the experimental animals, the distribution of stained cells was to some extent related to the underlying defects induced by the trauma. Cryosections of the traumatized but untransplanted control showed defects in lateral and dorsal fiber tracts rostral and caudal to the level of the injury (Fig. 1A). Typically, these defects are focal, variably shaped, low-density, sometimes cystic areas within white matter. Their irregular distribution suggests that they represent rostral or caudal extensions of the primary traumatic lesion.

Comparable defects have also been identified in specimens prepared for ultrastructural analysis (unpublished). In one case such a defect within dorsal column sensory tracts, examined 6 weeks after injury, was traced more than 1 cm *caudally* from the lesion site. This defect was found to contain fluid and macrophages and was surrounded by a cellular rim of tissue containing conspicuous astrocyte processes, replacing the ascending myelinated fiber tracts normally present in this location.

In the traumatized animals that received transplanted transgenic glia, incubation in the X-gal reaction solution revealed prominent clusters of LacZ$^+$ cells associated with equivalent regions showing focal defects in myelin staining, primarily in the dorsal and lateral white matter (Figs. 2–4). Such clusters were identified at multiple levels rostral and caudal to the injury site.

Stained cells were found not only in clusters associated with white matter defects but also scattered through the central gray (Figs. 2C, 3, and 4), sometimes in linear patterns, especially in regions of decussating fibers (Fig. 3A). Stained cells also formed conspicuous linear streaks within transversely sectioned white matter (Figs. 2B, 2G, and 3A). Otherwise, intact white matter regions showed relatively few stained cells in comparison with the adjacent gray matter (Figs. 3A and 4A). Dense, ovoid stained cells were also clearly associated with the margins of blood vessels (Fig. 5).

The morphology of the stained cells was difficult to assess critically in cryosections because of distortions introduced during tissue processing, which included detergent treatment as well as freezing. Nevertheless, in transverse sections through white matter, it was possible to identify cellular processes arising from stained cell bodies (Fig. 3B) and running perpendicular to the axis of the fiber tract.

Horizontal sections through white matter were even more revealing. In this plane, it was possible to identify cells giving rise to elongated LacZ$^+$ processes, ~30–60 μm in length, running parallel to the fiber tract (Figs. 6A and 6B). The intensity of the stain varied along the length of these processes. In some cases, these cells appeared as doublets, suggesting that a single cell underwent cell division and that both daughter cells remained at that site without further migration. Occasional oblique (Fig. 6A) and transverse (Fig. 6B) processes also could be seen arising from the same cells.

In contrast to the cells bearing multiple parallel proceses within fiber tracts (Fig. 6), the small, dense, ovoid LacZ$^+$ cells within gray matter and those associated with the margins of blood vessels (Fig. 5) gave rise to few recognizable processes. Thus, we identified two types of LacZ$^+$ cells in these specimens.

DISCUSSION

Our results show LacZ$^+$ cells within the traumatized rat spinal cord after xenografting transgenically marked glia carrying the LacZ gene under control of the MBP promoter into the site of experimental spinal cord lesions.

Despite the rigors of the preparative and staining procedures used for demonstration of X-gal reaction product, we were able to identify stained cells within fiber tracts that gave rise to multiple, relatively straight LacZ$^+$ processes running parallel to the nerve fibers. These process-bearing cells resemble differentiated oligodendrocytes, as shown in previous *in vivo* studies (28), and correspond to the type II oligodendrocytes described by Río Hortega (reviewed in ref. 25).

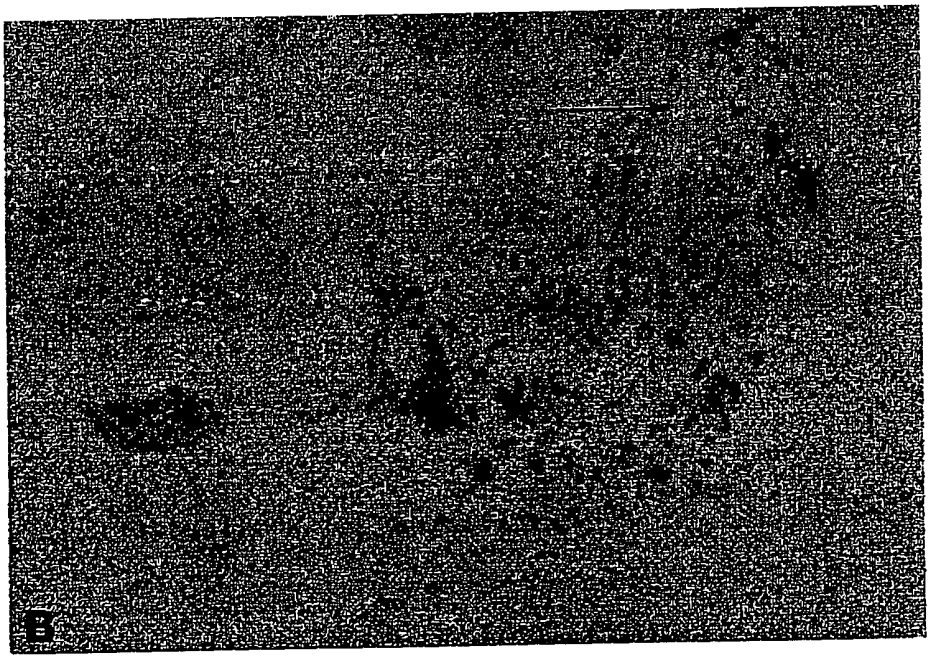

FIG. 8. (A) Detail showing clusters of stained cells, each surrounded by a lucent area of defective myelin (arrows) within lateral and dorsal column white matter. Stained cells are also scattered through gray matter and form linear strings within the central gray and within the lateral white matter at lower left. Same level as Fig. 2B. (B) Detail of Fig. 2F showing clusters of stained cells within central and dorsal horn gray matter and in white matter defects in dorsal and lateral white columns. Arrow indicates one such defect and, beyond it, a stained cell bearing a process.